Please amend the specification as follows:

Please replace the paragraph beginning at page 2, line 7, with the following paragraph:

Although the ATP-dependent ligases and NAD⁺-dependent ligases share little sequence homology, all the ligases investigated so far use the same KXDG (SEQ. ID. No. 24) motif to form adenylated enzyme intermediate (Tomkinson, et al., Bioessays, 19(10):893-901 (1997), Shuman, et al., Virology, 211(1):73-83 (1995), and Luo, et al., Nucleic Acids Res, 24(15):3079-3085 (1996)). Furthermore, they seem to be organized by similar domains and structural folds ((Doherty, et al., Nucleic Acids Res, 24(12):2281-2287 (1996), Subramanya, et al., Cell, 85(4):607-615 (1996), and Sekiguchi, et al., Nucleic Acids Res, 25(4):727-734 (1997)). The diversity of ligase sequences is not only reflected by their different optimal reaction conditions and kinetic rates, but more importantly by their different specificities toward match and mismatch substrates. Among the viral ATP-dependent ligases, the broad substrate tolerance is represented by the T4 enzyme which seals various mismatches on both the 3' and 5' side of the nick junction (Wu, et al., Gene, 76(2):245-254 (1989)). Vaccinia ligase ligates various mismatches at both 3'-hydroxyl or 5'-phosphate sides with the exception of purine-purine mismatch pairs at the 3'-hydroxyl side (Shuman, S., Biochemistry, 34(49):16138-161475 (1995)). Mammalian ATP-dependent ligases show different substrate sensitivity, as ligase I is more sensitive to 3' mismatches than ligase III (Husain, et al., J Biol Chem, 270(16):9683-9690 (1995)). Additionally, both ligase I and III tolerate a 3'C/T mismatch more than a 3'G/T mismatch. Little is known about archeal ATP-dependent ligases which may reveal the nature of the progenitor of ATP-dependent ligases. Studies on NAD⁺dependent DNA ligase from E. coli, along with T4 ligase, have contributed immensely to understanding of the basic biochemical pathway of the DNA ligation reaction (Lehman, I.R., Science, 186(4166):790-797 (1974) and Rossi, et al., Nucleic Acids Res, 25(11):2106-2113 (1997)). Studies on the NAD⁺-dependent ligase from *Thermus thermophilus* HB8 have revealed the highly discriminative power this enzyme possesses (Luo, et al., Nucleic Acids Res, 24(15):3071-3078 (1996)). Although mismatches at 5'-phosphate side are tolerated to some degree (5'A/C, 5'A/A, 5'C/A, 5'C/T, 5'G/T, 5'G/A, 5'T/T, 5'T/G), mismatches at the 3'hydroxyl side essentially abolish nick-closure activity except 3'G/T or 3'T/G mismatch (Luo, et al., Nucleic Acids Res, 24(15):3071-3078 (1996)). Apparently, sequence divergence and subsequent subtle structural variation among DNA ligases underlie an enzyme's recognition preferences toward different mismatched base-pairs.

Please replace the paragraph beginning at page 7, line 13, with the following paragraph:

Figure 1A-C show a sequence comparison of *Thermus* DNA ligases.

Figure 1A illustrates the evolutionary tree for *Thermus* DNA ligases. Figure 1B is a regional sequence alignment of nine *Thermus* ligases: *Tsp.* AK16D (SEQ. ID. No. 22); *Thermus* aquaticus YT-1 (SEQ. ID. No. 15); *Thermus Thermophilus* ("Tth") (SEQ. ID. No. 23);

Thermus flavus (SEQ. ID. No. 16); *Thermus filiformis* Tok4A2 (SEQ. ID. No. 17); *Thermus filiformis* Tok6A1 (SEQ. ID. No. 18); *Tsp.* SM32 (SEQ. ID. No. 19); *Tsp.* Vil3 (SEQ. ID. No. 20); *T. scot* (SEQ. ID. No. 21). The aa (i.e. amino acid) sequence of T. scot is retrieved from Genebank GenBank by accession number 1085749 (SEQ. ID. No. 31), is as follows:

<u>Met</u>	Thr	Leu	Glu	Glu	Ala	Arg	Lys	Arg	Val	Asn	Glu	Leu	Arg	Asp	Leu
1				5		•			10					15	
<u>Ile</u>	Arg	Tyr	His 20	Asn	Tyr	Arg	Tyr	Tyr 25	Val	Leu	Ala	Asp	Pro 30	Glu	Ile
			20		-										
Ser	Asp	Ala	Glu	Tyr	Asp	Arg	Leu	Leu	Arg	Glu	Leu	Lys	Glu	Leu	Glu
		35				_	40					45			
01	_			~ 1	. .	-		D	3		5	m1	01	01	T7 - 1
GIU	Arg 50	Phe	Pro	Glu	Leu	<u>ьуз</u> 55	Ser	Pro	Asp	Ser	Pro 60	Thr	Glu	Gin	val
Gly	Ala	Lys	Pro	Leu	Glu	Ala	Thr	Phe	Arg	Pro	Ile	Arg	His	Pro	Thr
65					70					75					80
											_		_	_	_ •
Arg	Met	Tyr	Ser		Asp	Asn	Ala	Phe	Asn 90	Phe	Asp	Glu	Leu	<u>Lys</u> 95	Ala
-				85					90		-			95	
Phe	Glu	Glu	Arg	Ile	Gly	Arg	Ala	Leu	Gly	Arg	Glu	Gly	Pro	Phe	Ala
			100					105					110		
_					_		_		_	_		_	_	_	_
Tyr	Thr	Val 115	Glu	His	Lys	Val	120	GLY	Leu	Ser	vai	125	Leu	Tyr	Tyr
		113					120					123			
Glu	Asp	Gly	Val	Leu	Val	Trp	Gly	Ala	Thr	Arg	Gly	Asp	Gly	Glu	Val
	130					135					140	-			
	Glu	Glu	Val	Thr		Asn	Leu	Leu	Thr		Pro	Thr	Ile	Pro	
145					150					155					160
Ara	Va]	Lys	Glv	Va)	Pro	Glu	Ara	Leu	Glu	Val	Ara	Glv	Glu	Val	Tvr
9		_, _	~- <u>1</u>	165		7-3	9		170		9	1		175	

Met	Pro	Ile	Glu 180	Ala	Phe	Leu	Arg	Leu 185	Asn	Glu	Glu	Leu	Glu 190	Glu	Lys
Gly	Glu	Lys 195	Ile	Phe	Lys	Asn	Pro 200	Arg	Asn	Ala	Ala	Ala 205	Gly	Ser	Leu
Arg	Gln 210	Lys	Asp	Pro	Arg	Ile 215	Thr	Ala	Arg	Arg	Gly 220	Leu	Arg	Ala	Thr
Phe 225	Tyr	Ala	Leu	Gly	Leu 230	Gly	Leu	Glu	Glu	Ser 235	Gly	Leu	Lys	Thr	Gln 240
Leu	Asp	Leu	Leu	His 245	Trp	Leu	Arg	Glu	Lys 250	Gly	Phe	Pro	Val	Glu 255	His
Gly	Phe	Ala	Arg 260	Ala	Glu	Gly	Ala	Glu 265	Gly	Val	Glu	Arg	11e 270	Tyr	Gln
Gly	Trp	Leu 275	Lys	Glu	Arg	Arg	Ser 280	Leu	Pro	Phe	Glu	Ala 285	Asp	Gly	Val
<u>Val</u>	Val 290	Lys	Leu	Asp	Glu	Leu 295	Ser	Leu	Trp	Arg	Glu 300	Leu	Gly	Tyr	Thr
<u>Ala</u>	Arg	Ala	Pro	Arg	Phe 310	Ala	Ile	Ala	Tyr	Lys 315	Phe	Pro	Ala	Glu	Glu 320
Lys	Glu	Thr	Arg	Leu 325	Leu	Gln	Val	Val	Phe 330		Val	Gly	Arg	Thr 335	Gly
Arg	Val	Thr	Pro 340	Val	Gly	Ile	Leu	Glu 345	Pro	Val	Phe	Ile	Glu 350	Gly	Ser
Val	Val	Ser 355	Arg	Val	Thr	Leu	His 360	Asn	Glu	Ser	Tyr	Ile 365	Glu	Glu	Leu
Asp	Val 370		Ile	Gly	Asp	Trp 375	Val	Leu	Val	His	Lys 380	Ala	Gly	Gly	<u>Val</u>
<u>Ile</u>	Pro	Glu	Val	Leu	Arg 390	Val	Leu	Lys	Glu	Lys 395	Arg	Thr	Gly	Glu	Glu 400
Arg															
	Pro	Ile	Arg	Trp 405	Pro	Glu	Thr	Cys	Pro 410	Glu	Cys	Gly	His	Arg 415	Leu
Val				405					410		Cys			415	
	Lys	Glu	Gly 420	405 Lys	Val	His	Arg	Cys 425	410 Pro	Asn		Leu	Cys 430	415 Pro	Ala

<u>Leu</u>	Val	Lys	Asp	Val	Ala 470	Asp	Leu	Tyr	Arg	Leu 475	Lys	Lys	Glu	Asp	Leu 480
Leu	Gly	Leu	Glu	Arg 485	Met	Gly	Glu	Lys	Ser 490	Ala	Gln	Asn	Leu	Leu 495	Arg
Gln	Ile	Glu	Glu 500	Ser	Lys	Gly	Arg	Gly 505	Leu	Glu	Arg	Leu	Leu 510	Tyr	Ala
Leu	Gly	Leu 515	Pro	Gly	Val	Gly	Glu 520	Val	Leu	Ala	Arg	Asn 525	Leu	Ala	Ala
His	Phe 530	Gly	Thr	Met	Asp	Arg 535	Leu	Leu	Glu	Ala	Ser 540	Leu	Glu	Glu	Leu
<u>Leu</u> 545	Gln	Val	Glu	Glu	Val 550	Gly	Glu	Leu	Thr	Ala 555	Arg	Gly	Ile	Tyr	Glu 560
Thr	Leu	Gln	Asp	Pro 565	Ala	Phe	Arg	Asp	Leu 570	Val	Arg	Arg	Leu	Lys 575	Glu
Ala	Gly	Val	Val 580	Met	Glu	Ala	Lys	Glu 585	Arg	Gly	Glu	Glu	Ala 590	Leu	Lys
Gly	Leu	Thr 595	Phe	Val	Ile	Thr	Gly 600	Glu	Leu	Ser	Arg	Pro 605	Arg	Glu	Glu
<u>Val</u>	Lys 610	Ala	Leu	Leu	Arg	Arg 615	Leu	Gly	Ala	Lys	Val 620	Thr	Asp	Ser	Val
<u>Ser</u>	Arg	Lys	Thr	Ser	Tyr 630	Leu	Val	Val	Gly	Glu 635	Asn	Pro	Gly	Ser	Lys 640
Leu	Glu	Lys	Ala	Arg 645	Ala	Leu	Gly	Val	Pro 650	Thr	Leu	Thr	Glu	Glu 655	Glu
Leu	Tyr	Arg	Leu 660	Ile	Glu	Glu	Arg	Thr 665	Gly	Lys	Pro	Val	Glu 670	Thr	Leu
Ala	Ser	_													

Ala Ser.

The adenylation motif KXDG (SEQ. ID. No. 24) is underlined and the adenylation site is marked by *. The numbering of aa is based on *Tsp.* AK16D ligase (SEQ. ID. No. 1.)

Figure 1C is a complete amino acid sequence of *Tsp.* AK16D ligase (SEQ. ID. No. 1). The adenylation motif KXDG (SEQ. ID. No. 24) is underlined and the adenylation site ¹¹⁸K is shown with a (*) above the residue. The complete sequence of *Tsp.* AK16D ligase gene and partial sequences of six other *Thermus* ligase genes have been deposited with GenBank under accession No. AF092862 for *Tsp.* AK16D (SEQ. ID. No. 1), AF092863 for *Thermus* aquaticus YT-1 (SEQ. ID. No. 25), as follows:

Pro 1	Glu	Leu	Lys	Ser 5	Pro	Asp	Ser	Pro	Thr	Glu	Gln	Val	Gly	Ala 15	Arg
Pro	Leu	Glu	Ser 20	Thr	Phe	Arg	Pro	Val 25	Arg	His	Pro	Thr	Arg 30	Met	Tyr
Ser	Leu	Asp 35	Asn	Ala	Phe	Ser	Leu 40	Asp	Glu	Val	Arg	Ala 45	Phe	Glu	Glu
Arg	Ile 50	Glu	Arg	Ala	Leu	Gly 55	Arg	Lys	Gly	Pro	Phe 60	Leu	Tyr	Thr	Val
Glu 65	His	Lys	Val	Asp	Gly 70	Leu	Ser	Val	Asn	Leu 75	Tyr	Tyr	Glu	Glu	Gly 80
Ile	Leu	Val	Phe	Gly 85	Ala	Thr	Arg	Gly	Asp 90	Gly	Glu	Thr	Gly	Glu 95	Glu
<u>Val</u>	Thr	Gln	Asn 100	Leu	Leu	Thr	Ile	Arg 105	Thr	Ile	Pro	Arg	Arg 110	Leu	Thr
Gly	Val	Pro 115	Asp	Arg	Leu	Glu	Val 120	Arg	Gly	Glu	Val	Tyr 125	Met	Pro	Ile
Glu	Ala 130	Phe	Leu	Arg	Leu	Asn 135	Gln	Glu	Leu	Glu	Glu 140	Ala	Gly	Glu	Arg
<u>Ile</u> 145	Phe	Lys	Asn	Pro	Arg 150	Asn	Ala	Ala	Ala	Gly 155	Ser	Leu	Arg	Gln	Lys 160
Asp	Pro	Arg	Val	Thr 165	Ala	Arg	Arg	Gly	Leu 170	Arg	Ala	Thr	Phe	Tyr 175	Ala
Leu	Gly	Leu	Gly 180	Leu	Glu	Glu	Thr	Gly 185	Leu	Lys	Ser	Gln	His 190	Asp	Leu
Leu	Leu	Trp 195	Leu	Lys	Glu	Arg	Gly 200	Phe	Pro	Val	Glu	His 205	Gly	Phe	Thr
Arg	Ala 210	Leu	Gly	Ala	Glu	Gly 215	Val	Glu	Glu	Val	Tyr 220	Gln	Ala	Trp	Leu
Lys 225	Glu	Arg	Arg	Lys	Leu 230	Pro	Phe	Glu	Ala	Asp 235	Gly	Val	Val	Val	Lys 240
Leu	Asp	Asp	Leu	Ala 245	Leu	Trp	Arg	Glu	Leu 250	Gly	Tyr	Thr	Ala	Arg 255	Ala
Pro	Arg	Phe	Ala 260	Leu	Ala	Tyr	Lys	Phe 265	Pro	Ala	Glu	Glu	Lys 270	Glu	Thr
Arg	Leu	Leu 275	Ser	Val	Ala	Phe	Gln 280	Val	Gly	Arg	Thr	Gly 285	Arg	Ile	Thr

Pro		Gly	Val	Leu	Glu	Pro 295	Val	Phe	Ile	Glu	Gly 300	Ser	Glu	Val	Ser
	290					295		 			300				
	Val	Thr	Leu	His		Glu	Ser	Phe	Ile		Glu	Leu	Asp	Val	Arg
<u>305</u>					310					315					320
Ile	Gly	Asp	Trp	Val	Leu	Val	His	Lys	Ala	Gly	Gly	Val	Ile	Pro	Glu
				325					330					335	
V = 1	T.211	Δrα	Val	T.211	Luc	Glu	Δra	Δνα	Thr	Glv	Glu	Glu	Lvs	Pro	Tle
vai	пец	Arg	340	ьец	цуз	Giu	ALG	345	1111	GIY	Gru	GIU	350	110	116
	_			_		_		_						_	
Leu	Trp	<u>Pro</u> 355	Glu	Asn	Cys	Pro	360	Cys	GLy	His	Ala	<u>Leu</u> 365	Leu	Lys	Glu
		333					300					303			
Gly		Val	His	Arg	Cys		Asn	Pro	Leu	Cys		Ala	Lys	Arg	Phe
	370					375					380				
Glu	Ala	Ile	Arg	His	Tyr	Ala	Ser	Arg	Lys	Ala	Met	Asp	Ile	Gln	
385					390					395					400
Leu	Glv	Glu	Lys	Leu	Ile	Glu	Lvs	Leu	Leu	Glu	Lvs	Glv	Leu	Val	Ara
			2 -	405					410				- i- i - i -	415	<u></u>
7 an	57-3	71.	7.00	T 011	Tire	7\ rc cr	T 011	7 22 22	Two	C1.1	7 cn	T 011	Lou	7 cn	LOU
ASP	vai	Ата	Asp 420	ьец	ıyı	ALG	ьеu	425	тÃ2	GIU	ASP	ьеи	430	ASP	ьец
Glu	Arg	Met 435	Gly	Glu	Lys	Ser	Ala 440	Glu	Asn	Leu	Leu	Arg 445	Gln	Ile	Glu
		433					440								
Glu		Lys	Gly	Arg	Gly		Glu	Arg	Leu	Leu		Ala	Leu	Gly	Leu
	450					455					460				
Pro	Gly	Val	Gly	Glu	Val	Leu	Ala	Arg	Asn	Leu	Ala	Leu	Arg	Phe	Gly
465					470					475					480
His	Met	Asp	Arg	Leu	Len	Glu	Ala	Glv	Len	Glv	Asp	Leu	Leu	Glu	Val
		-110-р		485				<u> </u>	490					495	
61	G1	77-3	01	G1	T	m)	7.7	70	71-	T1 -	T	7	m1	T	T
GIU	етх	vai	Gly 500	GIU	Leu	THE	Ата	505	Ala	ire	шeu	ASII	510	ьеи	туѕ
Asp	Pro		Phe	Arg	Asp	Leu		Arg	Arg	Leu	Lys	Glu 525	Ala	Gly	
		515					520					727			_
A EO	0206	I .c. 7	ri	д	(6	SEO.	ID N	- 20	\ £	`a 11 a					
Aru	92804	FIOF	Therm	us jia	vus (s	SEQ.	ID. N	0. 20	<u>, as 1</u>	<u>onow</u>	<u>s:</u>				
	Phe	Pro	Glu		Lys	Ser	Pro	Asp		Pro	Thr	Glu	Gln		Gly
1				5					10					15	
Ala	Arg	Pro	Leu	Glu	Ala	Thr	Phe	Arg	Pro	Val	Arg	His	Pro	Thr	Arg
-			20					25				·	30		

Met	Tyr	Ser	Leu	Asp	Asn	Ala	Phe	Asn	Phe	Asp	Glu	Leu	Lys	Ala	Phe
		35					40					45			
Glu	Glu	Arg	Ile	Glu	Arg	Ala	Leu	Gly	Arg	Glu	Gly	Pro	Phe	Ala	Tyr
	50					55					60				
Thr	Val	Glu	His	Lys	Val	Asp	Gly	Leu	Ser	Val	Asn	Leu	Tyr	Tyr	Glu
65					70					75					80
Asp	Glv	Val	Leu	Val	Tyr	Gly	Ala	Thr	Arq	Glv	Asp	Glv	Glu	Val	Gly
				85					90		_			95	
Glu	Glu	Val	Thr	Gln	Asn	Leu	Len	Thr	Tle	Pro	Thr	Tle	Pro	Ara	Ara
			100					105					110	9	9
T. 211	T.vs	Glv	Va 1	Pro	Glu	Arg	T. - 11	Glu	Val	Ara	Glv	Glu	Val	Tur	Met
<u></u>	шуо	115		110	Ora	1119	120	Oru	Vai	9	<u>OTY</u>	125	<u>var</u>	<u> </u>	1100
Pro	Val	Glu	Δla	Phe	T.011	Arg	T. 211	Δen	Glu	Glu	T.011	Glu	Glu	Δra	Glv
110	130	Oiu	AIG	1110	<u>пе и</u>	135	неш	ASII	Gra	Olu	140	Oru	Ora	Arg	<u>Oly</u>
70.1 -	7 20	T10	Pho	Tuc	7 cn	Dro	7 2 2	7 cn	7.1.5	7.1.5	7.1.5	C111	Sor	T 011	7, 20
145	AIG	тте	Pile	гуѕ	150	Pro	AIG	ASII	Ата	155	Ala	Gly	ser	ьец	160
	T	7	D	7	- 1-	m1		T	7	G1	T	70	n 1 -	ml	Dl
GIN	ьys	Asp	Pro	165	TTE	Thr	Ата	гуѕ	170	GIA	Leu	Arg	Ala	175	Pne
		_		_		_									
Tyr	Ala	Leu	180	Leu	GLY	Leu	GLu	185	Val	Glu	Arg	GIu	190	Val	Ala
Thr	Gln	Phe 195	Ala	Leu	Leu	His	7rp 200	Leu	Lys	Glu	Lys	<u>Ser</u> 205	Phe	Pro	Val
Glu	His 210	Gly	Tyr	Ala	Arg	Ala 215	Val	Gly	Ala	Glu	Gly 220	Val	Glu	Ala	<u>Val</u>
							·			•					
Tyr	Gln	Asp	Trp	Leu	Lys	Lys	Arg	Arg	Ala	Leu	Pro	Phe	Glu	Ala	Asp 240
223					230		··· -·			233			-		240
Gly	Val	Val	Val		Leu	Asp	Glu	Leu		Leu	Trp	Arg	Glu		Gly
				245					.250					255	
Tyr	Thr	Ala		Ala	Pro	Arg	Phe		Ile	Ala	Tyr	Lys		Pro	Ala
			260					265					270		
Glu	Glu	Lys	Glu	Thr	Arg	Leu	Leu	Asp	Val	Ala	Phe	Gln	Val	Gly	Arg
		275					280	-				285			
Thr	Gly	Arg	Val	Thr	Pro	Val	Gly	Ile	Leu	Glu	Pro	Val	Phe	Leu	Glu
	290					295					300				
Gly	Ser	Glu	Val	Ser	Arg	Val	Thr	Leu	His	Asn	Glu	Ser	Tyr	Ile	Glu
305					310					315			-		320

Glu	Leu	Asp	Ile	Arg 325	Ile	Gly	Asp	Trp	Val 330	Leu	Val	His	Lys	Ala 335	Gly
Gly	Val	Ile	Pro 340	Glu	Val	Leu	Arg	Val 345	Leu	Lys	Glu	Arg	Arg 350		Gly
Glu	Glu	Arg 355	Pro	Ile	Arg	Trp	Pro 360	Glu	Thr	Cys	Pro	Glu 365	Cys	Gly	His
Arg	Leu 370	Leu	Lys	Glu	Gly	Lys 375	Val	His	Arg	Cys	Pro 380	Asn	Pro	Leu	Cys
Pro 385	Ala	Lys	Arg	Phe	Glu 390	Ala	Ile	Arg	His	Phe 395	Pro	Ser	Arg	Lys	Ala 400
Met	Asp	Ile	Gln	Gly 405	Leu	Gly	Glu	Lys	Leu 410	Ile	Glu	Arg	Leu	Leu 415	Glu
Lys	Gly	Leu	Val 420	Lys	Asp	Val	Ala	Asp 425	Leu	Tyr	Arg	Leu	Arg 430	Lys	Glu
Asp	Leu	Val 435	Gly	Leu	Glu	Arg	Met 440	Gly	Glu	Lys	Ser	Ala 445	Gln	Asn	Leu
Leu	Arg 450	Gln	Ile	Glu	Glu	Ser 455	Lys	Arg	Arg	Gly	Leu 460	Glu	Arg	Leu	Leu
Tyr 465	Ala	Leu	Gly	Leu	Pro 470	Gly	Val	Gly	Glu	Val 475	Leu	Ala	Arg	Asn	Leu 480
Ala	Ala	Arg	Phe	Gly 485	Asn	Met	Asp	Arg	Leu 490	Leu	Glu	Ala	Ser	Leu 495	Glu
Glu	Leu	Leu	Glu 500	Val	Glu	Glu	Val	Gly 505	Glu	Leu	Thr	Ala	Arg 510	Ala	<u>Ile</u>
<u>Leu</u>	Glu	Thr 515	Leu	Lys	Asp	Pro	Ala 520	Phe	Arg	Asp	Leu	Val 525	Arg	Arg	Leu
Lys	Glu 530	Ala	Gly	Val	Glu	Met 535	Glu	Ala	Lys	Glu	Lys 540	Gly	Gly	Glu	Ala
<u>Leu</u> 545	Lys	Gly	Leu	Thr	Phe 550	Val	Ile	Thr	Gly	Glu 555	Leu		<u>:</u> _		

AF092865 for Thermus filiformis Tok4A2 (SEQ. ID. No. 27), as follows:

Asp	Ser	Pro	Thr	Glu	Gln	Val	Gly	Ala	Arg	Pro	Leu	Glu	Pro	Thr	Phe
1				5					10					15	
Arg	Pro	Val	Arg	His	Pro	Thr	Arg	Met	Tyr	Ser	Leu	Asp	Asn	Ala	Phe
			20					25					30		

Thr	Tyr	Glu 35	Glu	Val	Leu	Ala	Phe 40	Glu	Glu	Arg	Leu	Asp 45	Arg	Ala	Leu
Gly	Arg 50	Lys	Arg	Pro	Phe	Leu 55	Tyr	Thr	Val	Glu	His 60	Lys	Val	Asp	Gly
Leu 65	Ser	Val	Asn	Leu	Tyr 70	Tyr	Glu	Glu	Gly	Val 75	Leu	Val	Phe	Gly	Ala 80
Thr	Arg	Gly	Asp	Xaa 85	Xaa	Xaa	Xaa	Xaa	Xaa 90	Xaa	Xaa	Xaa	Xaa	Xaa 95	Xaa
Thr	Ile	Pro	Thr 100	Ile	Pro	Arg	Arg	Leu 105	Lys	Gly	Val	Pro	Asp 110	Arg	Leu
Glu	Val	Arg 115	Gly	Glu	Val	Tyr	Met 120	Pro	Ile	Glu	Ala	Phe	Leu	Arg	Leu
Asn	Glu 130	Glu	Leu	Glu	Glu	Arg 135	Gly	Glu	Lys	Val	Phe	Lys	Asn	Pro	Arg
Asn 145		Ala	Ala	Gly	Ser 150	Leu	Arg	Gln	Lys	Asp 155	Pro	Arg	Val	Thr	Ala 160
Lys	Arg	Gly	Leu	Arg 165	Ala	Thr	Phe	Tyr	Ala 170	Leu	Gly	Leu	Gly	Leu 175	Glu
Glu	Ser	Gly	Leu 180	Lys	Ser	Gln	Tyr	Glu 185	Leu	Leu	Leu	Trp	Leu 190	Lys	Glu
Lys	Gly	Phe 195	Pro	Val	Glu	His	Gly 200	Tyr	Glu	Lys	Ala	Leu 205	Gly	Ala	Glu
Gly	Val 210	Glu	Glu	Val	Tyr	Gln 215	Ala	Xaa	Xaa	Xaa	Lys 220	Arg	His	Ala	Leu
Pro 225	Phe	Glu	Ala		Gly 230	Val	Val	Val		Met 235	Asp	Asp	Leu		Leu 240
Trp	Gly	Glu	Leu	Gly 245	Tyr	Thr	Ala	Arg	Ala 250	Pro	Arg	Phe	Ala	Ile 255	Ala
Tyr	Lys	Phe	Pro 260	Ala	Glu	Glu	Asn	Glu 265	Thr	Arg	Leu	Leu	Asp 270	Val	Asp
Phe	Gln	Val 275	Gly	Arg	Thr	Gly	Arg 280	Val	Thr	Pro	Val	Gly 285	Ile	Leu	Glu
Pro	Val 290	Phe	Leu	Glu	Gly	Ser 295	Glu	Val	Ser	Arg	Val 300	Thr	Leu	His	Asn
Glu 305	Ser	Tyr	Ile	Glu	Glu 310	Leu	Asp	Ile	Arg	Ile 315	Gly	Asp	Trp	Val	Leu 320

Val His Lys Ala Gly Gly Val Ile Pro Glu Val Leu Arg Val Leu Lys Glu Arg Arg Thr Gly Glu Glu Arg Pro Ile Arg Trp Pro Glu Thr Cys Pro Glu Cys Gly His Arg Leu Leu Lys Glu Gly Lys Val His Arg Cys Pro Asn Pro Leu Cys Pro Ala Lys Arg Phe Glu Ala Ile Arg His Phe Pro Ser Arg Lys Ala Met Asp Ile Gln Gly Leu Gly Glu Lys Leu Ile Glu Arg Leu Leu Glu Lys Gly Leu Val Lys Asp Val Ala Asp Leu Tyr Arg Leu Arg Lys Glu Asp Leu Val Gly Leu Glu Arg Met Gly Glu Lys Ser Ala Gln Asn Leu Leu Arg Gln Ile Glu Glu Ser Lys Arg Arg Gly Leu Glu Arg Leu Leu Tyr Ala Leu Gly Leu Pro Gly Val Gly Glu Val Leu Ala Arg Asn Leu Ala Ala Arg Phe Gly Asn Met Asp Arg Leu Leu Glu Ala Ser Leu Glu Glu Leu Leu Glu Val Glu Val Gly Glu Leu Thr Ala Arg Ala Ile Leu Glu Thr Leu Lys Asp Pro Ala Phe Arg Asp Leu Val Arg Arg Leu Lys Glu Ala Gly Val Glu Met Glu Ala Lys Glu Lys Gly Gly Glu Ala Leu Lys Gly Leu Thr Phe Val Ile Thr Gly Glu Leu Ser

AF092866 for Thermus filiformis Tok6A1 (SEQ. ID. No. 28), as follows:

Arg	Phe	Pro	Glu	Phe	Lys	Ser	Pro	Asp	Ser	Pro	Thr	Glu	Gln	Val	Gly
1				5					10					15	
	-														
Ala	Arg	Pro	Leu	Glu	Pro	Thr	Phe	Arg	Pro	Val	Arg	His	Pro	Thr	Arg
			20					25					30	· · · · · · · · · · · · · · · · · · ·	

Met	Tyr		Leu	Asp	Asn	Ala		Thr	Tyr	Glu	Glu		Leu	Ala	Phe
		35		·			40					45			
Glu ——	Glu 50	Arg	Leu	Glu	Arg	Ala 55	Leu	Gly	Arg	Lys	Arg 60	Pro	Phe	Leu	Tyr
Thr 65	Val	Glu	His	Lys	Val	Asp	Gly	Leu	Ser	Val	Asn	Leu	Tyr	Tyr	Glu 80
Glu	Gly	Val	Leu	Val 85	Phe	Gly	Ala	Thr	Arg 90	Gly	Asp	Gly	Glu	Val 95	Gly
Glu	Glu	Val	Thr	Gln	Asn	Leu	Leu	Thr	Ile	Pro	Thr	Ile	Pro 110	Arg	Arg
Leu	Lys	Gly 115	Val	Pro	Asp	Arg	Leu 120	Glu	Val	Arg	Gly	Glu 125	Val	Tyr	Met
Pro	Ile 130	Glu	Ala	Phe	Leu	Arg 135	Leu	Asn	Glu	Glu	Leu 140	Glu	Glu	Arg	Gly
Glu 145	Lys	Val	Phe	Lys	Asn 150	Pro	Arg	Asn	Ala	Ala 155	Ala	Gly	Ser	Leu	Arg 160
Gln	Lys	Asp	Pro	Arg 165	Val	Thr	Ala	Lys	Arg 170	Gly	Leu	Arg	Ala	Thr 175	Phe
Tyr	Ala	Leu	Gly 180	Leu	Gly	Leu	Glu	Glu 185	Ser	Gly	Leu	Lys	Ser 190	Gln	Tyr
Glu	Leu	Leu 195	Leu	Trp	Leu	Lys	Glu 200	Lys	Gly	Phe	Pro	Val 205	Glu	His	Gly
Tyr	Glu 210	Lys	Ala	Leu	Gly	Ala 215	Glu	Gly	Val	Glu	Glu 220	Val	Tyr	Arg	Arg
Phe 225	Leu	Ala	Gln	Arg	His 230	Ala	Leu	Pro	Phe	Glu 235	Ala	Asp	Gly	Val	Val 240
<u>Val</u>	Lys	Leu	Asp	Asp 245	Leu	Ala	Leu	Trp	Arg 250	Glu	Leu	Gly	Tyr	Thr 255	Ala
Arg	Ala	Pro	Arg 260	Phe	Ala	Leu	Ala	Tyr 265	Lys	Phe	Pro	Ala	Glu 270	Glu	Lys
Glu	Thr	Arg 275	Leu	Leu	Asp	Val	Val 280	Phe	Gln	Val	Gly	Arg 285	Thr	Gly	Arg
<u>Val</u>	Thr 290	Pro	Val	Gly	Val	Leu 295	Glu	Pro	Val	Phe	Ile 300	Glu	Gly	Ser	Glu
<u>Val</u> 305	Ser	Arg	Val	Thr	Leu 310	His	Asn	Glu	Ser	Tyr 315	Ile	Glu	Glu	Leu	Asp 320
Ile	Arg	Ile	Gly	Asp	Trp	Val	Leu	Val	His	Lys	Ala	Gly	Gly	Val	Ile

	325	330	335
Pro Glu Val Leu		Lys Glu Arg Arg Thr 345	Gly Glu Glu Arg 350
Pro Ile Arg Trp	Pro Glu Thr	Cys Pro Glu Cys Gly 360	His Arg Leu Val
Lys Glu Gly Lys	Val His Arg 375	Cys Pro Asn Pro Leu 380	Cys Pro Ala Lys
Arg Phe Glu Ala	Ile Arg His 390	Tyr Ala Ser Arg Lys 395	Ala Met Asp Ile 400
Glu Gly Leu Gly	Glu Lys Leu 405	Ile Glu Arg Leu Leu 410	Glu Lys Gly Leu 415
Val Arg Asp Val		Tyr His Leu Arg Lys 425	Glu Asp Leu Leu 430
Gly Leu Glu Arg	Met Gly Glu	Lys Ser Ala Gln Asn 440	Leu Leu Arg Gln
Ile Glu Glu Ser 450	Lys His Arg 455	Gly Leu Glu Arg Leu 460	Leu Tyr Ala Leu
Gly Leu Pro Gly	Val Gly Glu 470	Val Leu Ala Arg Asn 475	Leu Ala Arg Arg 480
Phe Gly Thr Met	Asp Arg Leu 485	Leu Glu Ala Ser Leu 490	Glu Glu Leu Leu 495
Glu Val Glu Glu 500		Leu Thr Ala Arg Ala 505	Ile Leu Glu Thr 510
Leu Lys Asp Pro	Ala Phe Arg	Asp Leu Val Arg Arg 520	Leu Lys Glu Ala 525
Gly Val Ser Met	Glu Ser Lys 535	Glu Glu	
AF092867 for <i>Tsp</i> . V	Vil3 (SEQ. ID. No	o. 29), as follows:	
	Ser Pro Thr	Glu Gln Val Gly Ala	
	Pro Ile Arg	His Pro Thr Arg Met	
		Val Arg Thr Phe Glu	
35		40	45

Arg	Ala 50	Leu	Gly	Arg	Lys	Gly 55	Pro	Phe	Val	Tyr	Thr 60	Val	Glu	His	Lys
Val 65	Asp	Gly	Leu	Ser	Val	Asn	Leu	Tyr	Tyr	Glu 75	Glu	Gly	Ile	Leu	Val 80
Trp	Gly	Ala	Thr	Arg 85	Gly	Asp	Gly	Glu	Thr 90	Gly	Glu	Glu	Val	Thr 95	Gln
Asn	Leu	Leu	Thr	Ile	Pro	Thr	Ile	Pro	Arg	Arg	Leu	Lys	Gly 110	Val	Pro
Glu	Arg	Leu 115	Glu	Val	Arg	Gly	Glu 120	Val	Tyr	Met	Pro	Ile 125	Glu	Ala	Phe
Leu	Arg 130	Leu	Asn	Glu	Glu	Leu 135	Glu	Glu	Lys	Gly	Glu 140	Lys	Ile	Phe	Lys
<u>Asn</u> 145	Pro	Arg	Asn	Ala	Ala 150	Ala	Gly	Ser	Phe	Arg 155	Gln	Lys	Asp	Pro	Arg 160
<u>Ile</u>	Thr	Ala	Arg	Arg 165	Gly	Leu	Arg	Ala	Thr 170	Phe	Tyr	Ala	Leu	Gly 175	Leu
Gly	Leu	Glu	Glu 180	Ser	Gly	Leu	Lys	Thr 185	Gln	Leu	Asp	Leu	Leu 190	His	Trp
Leu	Arg	Glu 195	Lys	Gly	Phe	Pro	Val 200	Glu	His	Gly	Phe	Ala 205	Arg	Ala	Glu
Gly	Ala 210	Glu	Gly	Val	Glu	Arg 215	Ile	Tyr	Gln	Gly	Trp 220	Leu	Lys	Glu	Arg
Arg 225	Ser	Leu	Pro	Phe	Glu 230	Ala	Asp	Gly	Val	Val 235	Val	Lys	Leu	Asp	Glu 240
Leu	Ser	Leu	Trp	Arg 245		Leu	Gly	Tyr	Thr 250		Arg	Ala	Pro	Arg 255	Phe
Ala	Ile	Ala	Tyr 260	Lys	Phe	Pro	Ala	Glu 265	Glu	Lys	Glu	Thr	Ala 270	Leu	Phe
Gln	Val	Val 275	Leu	Gln	Val	Gly	Arg 280	Thr	Gly	Gln	Val	Thr 285	Pro	Val	Gly
<u>Ile</u>	Leu 290	Glu	Pro	Val	Phe	Ile 295	Glu	Gly	Ser	Glu	Val 300	Ser	Arg	Val	Thr
<u>Leu</u> 305	His	Asn	Glu	Ser	Tyr 310	Ile	Glu	Asp	Leu	Asp 315	Val	Arg	Ile	Gly	Glu 320
Trp	Val	Leu	Val	His 325	Asn	Ala	Gly	Gly	Val 330	Ile	Pro	Glu	Val	Leu 335	Arg
Val	Leu	Lys	Glu	Lys	Arg	Thr	Gly	Glu	Glu	Arg	Pro	Ile	Arg	Trp	Pro

	340	3	45	350
Glu Thr Cys 1	Pro Glu Cy	s Gly His A 360	arg Leu Val Lys	Glu Gly Lys Val
His Arg Cys 1	Pro Asn Pr	o Leu Cys P 375	Pro Ala Lys Arg	Phe Glu Ala Ile
Arg His Tyr A	Ala Ser Ar 39		Met Asp Ile Gly 395	Gly Leu Gly Glu 400
Lys Leu Ile (Glu Lys Le 405	u Leu Glu L	ys Gly Leu Val 410	Lys Asp Val Ala 415
	Arg Leu Ly 420		sp Leu Val Gly 25	Leu Glu Arg Met
Gly Lys Lys S	Ser Ala Gl	n Asn Leu L 440	eu Arg Gln Ile	Glu Lys Ser Lys 445
Ala Arg Gly 1	Leu Glu Ar	g Leu Leu T 455	yr Ala Leu Gly 460	Leu Pro Gly Val
Gly Glu Val 1 465	Leu Ala Ar 47		ala Ala His Phe 475	Gly Thr Met Asp
Arg Leu Leu (Glu Ala Se 485	r Leu Glu G	lu Leu Leu Gln 490	Val Glu Glu Val 495
Gly Glu Leu	Thr Ala Ar 500		<u>'yr</u> 05;	
and AF092868 fo	or <i>Tsp</i> . SM32	(SEQ. ID. No.	30), as follows:	
Asp Asn Ala I	Phe Thr Hi	s His Asp L	eu Lys Ala Phe	Glu Asp Arg Val
		g Glu Gly P		Thr Val Glu His
Lys Val Asp 0				Glu Gly Ile Leu
	Ala Pro Ar		ly Glu Val Gly 60	Glu Glu Val Thr
	Leu Thr Il	e Pro Thr I		Leu Lys Gly Val
Pro Glu Arg I	Leu Glu Va 85	l Arg Gly G	lu Val Tyr Met	Pro Ile Glu Ala 95

Phe	Leu	Arg	Leu 100	Asn	Glu	Glu	Leu	Glu 105	Glu	Ala	Gly	Glu	Lys 110	Val	Phe
Lys	Asn	Pro 115	Arg	Asn	Ala	Ala	Ala 120	Gly	Ser	Leu	Arg	Gln 125	Lys	Asp	Pro
Arg	Ile 130	Thr	Ala	Lys	Arg	Gly 135	Leu	Arg	Ala	Thr	Phe 140	Tyr	Ala	Leu	Gly
<u>Leu</u>	Gly	Leu	Glu	Glu	Ser 150	Gly	Leu	Lys	Thr	Gln 155	Tyr	Glu	Phe	Leu	Leu 160
Trp	Phe	Lys	Glu	Lys 165	Gly	Phe	Pro	Val	Glu 170	His	Gly	Phe	Ala	Arg 175	Ala
Thr	Gly	Ala	Glu 180	Gly	Val	Glu	Arg	Val 185	Tyr	Gln	Gly	Trp	Leu 190	Gln	Lys
Arg	Arg	Lys 195	Leu	Pro	Phe	Glu	Ala 200	Asp	Gly	Val	Val	Val 205	Lys	Leu	Asp
Glu	Leu 210	Ala	Leu	Trp	Arg	Glu 215	Leu	Gly	Tyr	Thr	Ala 220	Arg	Ala	Pro	Arg
Phe 225	Ala	Ile	Ala	Tyr	Lys 230	Phe	Pro	Ala	Glu	Glu 235	Lys	Glu	Thr	Arg	Leu 240
-						-									
	Asp	Val	Val	Phe 245		Val	Gly	Arg	Thr 250		Arg	Val	Thr	Pro 255	Val
Leu				245	Gln				250	Gly					
Leu	Ile	Leu	Glu 260	245 Pro	Gln Val	Leu	Ile	Glu 265	250 Gly	Gly	Glu	Val	Ser 270	255	Val
Leu Gly Thr	Ile	Leu His 275	Glu 260 Asn	Pro Glu	Gln Val Ser	Leu	Ile Ile 280	Glu 265 Glu	Gly Glu	Gly Ser Leu	Glu	Val Ile 285	Ser 270 Arg	255 Arg	Val Gly
Gly Thr	Ile Leu Trp 290	Leu His 275 Val	Glu 260 Asn	Pro Glu	Gln Val Ser	Leu Tyr Lys 295	Ile Ile 280 Ala	Glu 265 Glu Gly	Glu Gly	Ser Leu Val	Glu Asp Ile 300	Val Ile 285 Pro	Ser 270 Arg	255 Arg Ile Val	Val Gly
Gly Thr Asp Arg 305	Ile Leu Trp 290 Val	Leu His 275 Val	Glu 260 Asn Leu	Pro Glu Val Glu	Gln Val Ser His	Leu Tyr Lys 295 Arg	Ile Ile 280 Ala Thr	Glu 265 Glu Gly	Glu Gly Ala	Ser Leu Val Glu 315	Asp Ile 300 Arg	Val Ile 285 Pro	Ser 270 Arg Glu	255 Arg Ile Val	Val Gly Leu Trp 320
Leu Gly Thr Asp Arg 305	Ile Leu Trp 290 Val	Leu His 275 Val Leu	Glu 260 Asn Leu Lys	Pro Glu Val Glu Pro 325	Gln Val Ser His Arg 310 Glu	Leu Tyr Lys 295 Arg	Ile 280 Ala Thr	Glu 265 Glu Gly His	Glu Gly Ala His 330	Ser Leu Val Glu 315 Leu	Glu Asp Ile 300 Arg	Val Ile 285 Pro Pro	Ser 270 Arg Glu Ile	Ile Val Val	Caly Leu Trp 320 Lys
Leu Gly Thr Asp Arg 305 Pro	Ile Leu Trp 290 Val Glu	Leu His 275 Val Leu Asn	Glu 260 Asn Leu Lys Cys	Pro Glu Val Glu Pro 325 Pro	Ser His Arg 310 Glu Asn	Leu Tyr Lys 295 Arg Cys	Ile 280 Ala Thr Gly Leu	Glu 265 Glu Gly His Cys 345	Gly Gly Ala His 330 Pro	Ser Leu Val Glu 315 Leu Ala	Glu Asp Ile 300 Arg Val	Val Ile 285 Pro Pro Lys	Ser 270 Arg Glu Ile Glu Phe 350	Ile Val Gly 335	Ual Gly Leu Trp 320 Lys

Ala	Asp	Leu	Tyr	Arg	Leu	Arg	Lys	Glu	Asp	Leu	Val	Gly	Leu	Glu	Arg
385					390					395					400
Met	Gly	Glu	Lys	Ser	Ala	Glu	Asn	Leu	Leu	Arg	Gln	Ile	Glu	Glu	Ser
				405					410					415	
Lys	His	Arg	Gly	Leu	Glu	Arg	Leu	Leu	Tyr	Ala	Leu	Gly	Leu	Pro	Gly
			420					425					430	-	
										_					
Val	Gly	Glu	Val	Leu	Ala	Arg	Asn	Leu	Ala	Ala	Arg	Phe	Gly	Thr	Met
		435					440				-	445			
					_										
Asp	Arg	Leu	Leu	Glu	Ala	Thr	Leu	Glu	Glu	Leu	Leu	Glu	Val	Glu	Glu
	450				_	455					460				
					_										
Val	Gly	Glu	Leu	Thr	Ala	Arg	Gly	Ile	Trp	Glu	Thr	Leu	Gln	Asp	Pro
465					470					475					480
<u>Ala</u> .	•														

Please replace the paragraph beginning at page 10, line 25, with the following paragraph:

The thermostable ligase of the present invention is also characterized by having an arginine adjacent to the active site lysine (i.e. K) in the KXDG (SEQ. ID. No. 24) motif (where X is any amino acid).

Please replace the paragraph beginning at page 26, line 3, with the following paragraph:

The oligonucleotide perfect match substrate was formed by annealing two short oligonucleotides (33-mer for LP3'C (SEQ. ID. No. 11) and 30-mer for Com3F (SEQ. ID. No. 12)) with a 59-mer complementary oligonucleotide (Glg). Oligonucleotides LP3'C and Glg (SEQ. ID. No. 14) were in 1.5-fold excess so that the all the 3' Fam labeled Com3F represented nicked substrates (see Luo, et al., Nucleic Acids Res, 24(15):3071-3078 (1996), which is hereby incorporated by reference). The T/G mismatch substrate was formed by annealing LP3'T (SEQ. ID. No. 13), which introduced a single base-pair mismatch at the 3'-end of the nick junction, along with Com 3'F to the complementary strand (Glg). The nicked DNA duplex substrates were formed by denaturing DNA probes at 94°C for 2 min followed by re-annealing at 65°C for 2 min in ligation buffer. The sequences of the oligonucleotides were listed below (p represents 5' phosphate group):

(SEQ. ID. No. 12) pAGTTGTCATAGTTTGATCCTCTAGTCTGGG-FAM-3' Com3
LP3'T (SEQ. ID. No. 13) 5'- CCCTGTTCCAGCGTCTGCGGTGTTGCGTT
LP3'C (SEQ. ID. No. 11) 5'-AAACCCTGTTCCAGCGTCTGCGGTGTTGCGTC
Glg (SEQ. ID. No. 14) 3'-GGGACAAGGTCGCAGACGCCACAACGCAGTCAACAGTATCAAACTAGGAGATCAGACCC-5'

Please replace the paragraph beginning at page 27, line 15, with the following paragraph:

Amino acid sequence alignment of five Gram negative bacterial NAD⁺dependent DNA ligases indicates that Tth ligase is 93% identical to Thermus scotoductus ligase, 49% to Rhodothermus marinus ligase, 48% to E. coli ligase, and 38% to Zymomonas mobilis based on sequence data retrieved from GeneBank. Degenerate primers corresponding to highly conserved regions of these ligases were used to amplify fragments of ligase genes from seven Thermus strains which represent a worldwide collection: Thermus flavus from Japan (SEQ. ID. No. 16), Thermus aquaticus YT-1 (SEQ. ID. No. 15) and Thermus sp. AK16D from Yellowstone National Park in the United States (SEQ. ID. No. 22), Thermus filiformis Tok4A2 (SEO. ID. No. 17) and Thermus filiformis Tok6A1 (SEO. ID. No. 18) from New Zealand, Thermus sp. SM32 (SEQ. ID. No. 19) from Azores, and Thermus sp. Vil3 (SEQ. ID. No. 20) from Portugal. The sequences of amplified ligase fragments ranging from 1.4 to 1.6 kb were determined by directly sequencing the PCR products using an ABI 373 automated sequencer. Thermus ligases, in general, were highly conserved during evolution as demonstrated by 85%-98% sequence identity. In contrast, the amino acid sequences of the restriction endonuclease TaqI and its isoschizomers from the identical strains show only 50-70% aa identities (Cao, et al., Gene, 197:205-214 (1997), which is hereby incorporated by reference). Thermus ligases in general show 30-40% sequence identities as compared with DNA ligases from other bacteria. The sequence divergence is slightly higher among the different geographic groups than within the same group, which may reflect random drift or adaptation to their respective local environments (Figure 1). Thermus flavus, Thermus filiformis Tok4A2, Thermus filiformis Tok6A1, Thermus sp. SM32, Thermus sp. Vil3, Thermus aquaticus YT-1, and Thermus sp. AK16D (SEQ. ID. No. 14 1) ligases shared 98.2%, 89.9%, 89.5%, 89.8%, 88.3%, 88.2%, 88.1% with Thermus thermophilus HB8 DNA ligase, respectively. The adenylation site of the enzymes (118KXDG (SEQ. ID. No. 24) where X is in general a hydrophobic residue), as identified by site-directed mutagenesis of *Tth* DNA ligase, is completely identical among all *Thermus* ligases, furthermore, the flanking sequences of the adenylation motif are also identical except Tsp. AK16D in which the aa residues ¹¹⁷H before the ¹¹⁸K is substituted by an ¹¹⁸R (Figure 1B). In non-Thermus

NAD⁺-dependent ligases discovered to date, the corresponding position is either a Pro or a Leu. The two isolates from Japan can be distinguished from the other *Thermus* strains by a 3-aa-insertion at position 234.

Please replace the paragraph beginning at page 29, line 24, with the following paragraph:

Divalent metal ion is indispensable for each of the three steps in a ligation reaction: (i) adenylation of a lysine residue in the adenylation motif KXDG (SEQ. ID. No. 24); (ii) transfer of the adenylate to the 5' phosphate to form a DNA-adenylate intermediate; and (iii) formation of the phosphodiester bond with the release of adenosine monophosphate (AMP). In general, Mg²⁺ is the preferred metal ion for both ATP-dependent and NAD⁺dependent ligases. Mg²⁺ was substituted with alkaline earth metal ion Ca²⁺ and commonly studied period 4 transition metal ions. Tth and Tsp. AK16D ligases could use Mn²⁺ as an alternative metal cofactor to support ligation activity (Figure 4). Both enzymes were less active with Ca²⁺, while Co²⁺, Ni²⁺, Cu²⁺, and Zn²⁺ failed to support ligation. In comparison, ATP-dependent ligase from Hin (i.e. Haemophilus influenzae) uses only Mg²⁺ and Mn²⁺ as the metal cofactor for nick closure but not Ca²⁺, Co²⁺, Cu²⁺, and Zn²⁺ (Cheng, et al., Nucleic Acids Res, 25(7):1369-1374 (1997), which is hereby incorporated by reference); ATPdependent ligase from Chlorella virus PBCV-1 can use Mg²⁺, Mn²⁺, and Co²⁺ but not Ca²⁺, Cu²⁺, and Zn²⁺ (Ho, et al., J Virol, 71(3):1931-1937 (1997), which is hereby incorporated by reference). Using Ca²⁺ as the metal cofactor, *Thermus* enzymes were able to convert most of the substrate into the DNA-adenylate intermediate. However, the rates of nick closure were reduced which led to the accumulation of the DNA-adenylate intermediate (Figure 4B). A small amount of the intermediate was observed with Ni²⁺; however, ligation product was not observed at the current detection level, suggesting that Ni²⁺ could not support the nick closure step (Figure 4B). To further compare the relative activity of the two Thermus ligases with Mg²⁺ and Mn²⁺, the generation of ligation product was first monitored over a 20-min time period. As shown in Figure 5, the *Thermus* enzymes were consistently more active with Mg²⁺ than with Mn²⁺. Second, ligation activity up to 40 mM Mg²⁺ or Mn²⁺ concentrations (Figure 6) was assayed. Both of the enzymes responded sensitively to the change of the metal ion concentration in the reaction mixture. At high M²⁺ concentrations, the high ionic strength may inhibit the enzyme activity, consistent with KCl dependence profile (Figure 4).

Similar to the time-course results, the *Thermus* enzymes were more active with Mg²⁺ than with Mn²⁺ (Figure 6). The discrepancy on the relative activity of *Thermus* ligases between this study and an earlier report may be due to use here of cloned enzymes while the earlier work used purified native enzyme (Takahashi, et al., <u>J Biol Chem</u>, 259(16):10041-10047 (1984), which is hereby incorporated by reference).

Please replace the paragraph beginning at page 35, line 24, with the following paragraph:

Studies on *Tth* DNA ligase has deepened understanding of thermostable ligases and has reaffirmed the common theme of ligation — adenylation of ligase at the KXDG (SEQ. ID. No. 24) motif (Luo, et al., Nucleic Acids Res, 24(15):3079-3085 (1996), which is hereby incorporated by reference). This study reveals that *Thermus* ligases may differ from each other as to substrate specificity despite their highly identical primary protein sequences. A highly homologous structure can be anticipated from various *Thermus* ligases, but subtle local environments may dictate the probability of accepting a particular mismatch as the substrate. The fidelity of the Thermus ligases may be determined by multiple domains, multiple motifs and/or multiple sequence elements. In comparison of Tth and Tsp. AK16D ligases, one can find that although K294R (in an identical local environment, see Figure 1B) enhances the fidelity of *Tth* ligase (Luo, et al., Nucleic Acids Res, 24(15):3071-3078 (1996), which is hereby incorporated by reference), Tsp. AK16D ligase with a K in this position can still demonstrate superior mismatch discrimination. Additional sequence elements remain to be uncovered. The R substitution at the adjacent position to the KXDG (SEQ. ID. No. 24) motif may have an effect on the Tsp. AK16D ligase's specificity, because studies on Chlorella ligase has emphasized the importance of occupying AMP binding pocket for nick recognition (Sriskanda, et al., Nucleic Acids Res, 26(2):525-531 (1998)). The accumulation of DNA-adenylate intermediate with some divalent metal ions by Tsp. AK16D ligase asserts that the nick closure step of a ligation reaction can be sensitive to the selection of metal ions, gapped substrates and mismatch substrates. More structural and functional studies on Tsp. AK16D ligase could reveal how this enzyme achieves high fidelity with different substrates and different metal ions.